GENITAL WARTS

by

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I N D E X.

GENITAL WARTS.

INTRODUCTION.

Genital Wart is the name chosen for the papillomata which occur on the genital regions of males and females. Due to their situation they are associated in a general way with venereal diseases. So that of the many names used to describe them, most, such as Gonorrhoeal Wart and Venereal Wart, suggest that they are a result of one of the venereal infections or at least that they are venereal in origin.

At one time they were thought to be a local complication of Gonorrhoea and even to be directly caused by the Gonococcus. They have also been confused with the Condylomata Lata of Syphilis. However, it is now becoming generally recognised that they have no connection with Gonorrhoea or Syphilis although they may frequently coexist with one or both of these conditions (Lees 1937 (26)). Also, though they may result from direct contact with an infected person, there are many cases when they are not venereal in origin. In spite of this, they are most commonly called Venereal Warts. It seems desirable to use a name which more exactly describes the condition/

condition. Therefore, if 'Wart' alone is insufficient, Genital may be used without giving a false impression of the etiology.

This confusion with regard to cause and name reflects the relatively little interest which appears to be taken in the condition. But, following the discoveries made by Shope (38) of transmissible papillomata in rabbits and their relation to the problems of new growths, Genital Warts have a new significance. They offer an opportunity of studying such papillomata in the human subject and they may form the link between these virus produced tumours of Shope, and the new growths in man.

A preliminary investigation into this condition was carried out in 1936 when it was possible to use the material available in the venereal diseases department of The Royal Infirmary, Edinburgh. At this time the clinical condition was studied, the presence of inclusion bodies was described and further possible means of approach to the problem were sought. Accordingly the present work is a continuation of that previously carried out. Other methods of demonstrating the presence of the virus have been investigated and further attempts have been made to transmit these papillomata to animals.

This/

This work was carried out while holding the appointment of Officer in charge of the Brigade Laboratory, Mhow, Central India. For these investigations the material was obtained from among the British and Indian troops and civilians in Mhow.

No special attempt was made to study the clinical condition. Among the civilian population these warts were considered so slight that cases only came under observation either when they were in hospital for some other cause or during routine medical examination for military employment. In this way nine cases in all were seen during 1937 and 1938. These provided sufficient material for the investigations.

Reliable statistics are available only from the British and Indian Military Hospitals. Four cases of Genital Warts were seen in these Hospitals during the two years 1937 and 1938. This gives an incidence of 21.5 cases of Genital Warts per 1000 venereal cases treated.

	Genital Warts	Syphilis	Gonorrhoea	Soft Sore	Lympho gran. Non Specific Urethritis etc.
British Troops	3	11	69	19	36
Indian Troops	1	26	13	3	5

Table showing the relative incidence of Venereal Diseases in British and Indian troops in 1937 and 1938.

DEFINITION AND CLINICAL TYPES.

The term Genital Wart is taken to include the papillomata which are found on the penis, the vulva and round the anus. These papillomata are usually, multiple pedunculated, cauliflower like outgrowths, with an irregular surface. They do not extent into the subcutaneous tissues. They form a convenient clinical group as for many years they have been considered a separate lesion and treated with the venereal diseases. Also the symptoms and complications to which they may give rise are due entirely to their anatomical position.

In the male they are usually present in the coronal sulcus but they may extend onto the glans penis and Frenum and onto the mucosal surface of the prepuce. They are also found round the anus where they tend to become flattened and difficult to distinguish from Condylomata lata. Rarely they may be present in the urethra. They show marked variation both in numbers and in size. A single wart may be present but this can be either little more than a thickening of the epithelial surface or a pedunculated mass of branching processes over two centimetres in diameter. More usually several small distinct/

Fig.1. Genital Wart from the coronal sulcus. X2.

distinct warts are present though the total number may be fifty or more.

This differs from the condition often found in female patients where the warts may coalesce and form a thick mass of branching processes covering the whole of the genital region. When the condition is less severe, only a few warts may be found. These may occur on both surfaces of the labia majora and minora, around the urinary meatus, clitoris and anus or in the vagina. As in the male the size and number of the warts varies considerably. This variation is most marked in the pregnant female where, even as early as the second month, they proliferate very rapidly, at times quickly covering the whole of the surface of the vulva.

The condition may be further modified in both sexes by an irritant discharge. The discharge may be gonorrhoeal in origin or non specific. Combining the previous series of cases with the present series, the following figures were obtained for males:-

Total no. of cases.	Those with gonorrhoea.	Those with nonspecific balanitis
24	8	6

Three/

Three pregnant females previously seen all had large growths of warts associated with a profuse, non-specific discharge.

The times of onset of the warts and the discharge did not appear to bear any constant relationship to each other. Histories were obtained of a balanitis present for some weeks before any discharge was noted. Other patients stated that the two conditions appeared about the same time. It is probable that the two are entirely separate but that they exert a modifying influence on each other.

The warts themselves may also become superficially infected. This may lead to ulceration and the formation of a slough.

The size of the warts seems to have no constant relation either to their age or to the presence of a discharge. Four small warts, from one to two millimetres in diameter, were found in the coronal sulcus of a man with a balanitis which had been present for some months. While two warts, the largest of which was almost two centimetres in diameter, were present in the coronal sulcus of a patient with no other signs of disease. In both cases the patient had only been aware of the warts for two or three months.

That the condition is infective and may be transmitted by contact is now generally accepted. Only three patients of the total number seen gave a definite/

definite history of intercourse with a person already suffering from the condition. All the other cases with four exceptions admitted a possible exposure to an infection of this type. It was difficult to elicit a definite incubation period but it appeared to be two or three months or less.

This, however, is not the only way in which Genital Warts may be caused. In 1900 Rasch first noted the simultaneous appearance of warts on both the hands and genitals. Seventy per cent of Brande's thirty-eight cases and fifty per cent of Frey's also had warts in both places. Auto infection from the hand may therefore occur although this seems relatively uncommon in the present series of cases where only four out of twenty seven patients had warts on other parts of the body.

The absence of warts on other parts of the body does not entirely rule out this means of infection as the following case illustrates.

A Madrasi cook aged 18 was found on routine medical examination to have a minute wart in the coronal sulcus. He stated that two years previously a friend had warts on his hands. Sometime later the patient developed a small wart on his right shoulder which disappeared spontaneously after about a year. Some three months later he first noticed

the wart in his coronal sulcus. He denied ever having had intercourse.

If they are left untreated, the warts may remain stationary for many months. But, for no apparent reason, they may suddenly increase both in size and numbers. The only definite factor which may cause these changes is pregnancy. The warts increase enormously during the first few months but after parturition they undergo involution and may even completely disappear without treatment.

The patients in the present series of cases did not report sick unless they had some other disease in addition to the genital warts. Therefore it was difficult to reach any conclusion as to the relative frequency of the condition among those with syphilis and gonorrhoea as opposed to those not suffering from these two diseases. The only reliable indication was that given by the figures for British and Indian troops in Mhow during 1937 and 1938. As only four cases of genital warts occurred during this period, no definite conclusions could be reached. But as all the troops are medically examined every month, it may be assumed that the figures given are accurate.

	No. of cases.	No. with genital warts.
Gonorrhoea	82	1
Syphilis	37	Nil.
Soft Sore	22	Nil.

(Table showing incidence of warts among venereal patients).

During this period four cases were seen. Therefore it does not appear that patients with gonorrhoea, syphilis or soft sore are more liable to have genital warts than any other class of patient.

ETIOLOGY AND PATHOLOGY.

In addition to the clinical observations of the simultaneous appearance of genital and common warts, the occurrence of laryngeal papillomata in association with flat warts of the skin was noted by Werner (1894) and Thost (1911). There is also considerable experimental evidence of the relationship between the various types of papillomata. Waelsch (1918) was able to produce flat warts on the skin and papillomata on mucus membranes by injecting ground up material from a 'fig wart'. A similar result was obtained by Serra (1924) who found that warts on the hand occurred after injection of a Chamberland filtrate of 'condylomata accuminata'. Also in 1923 Ullmann inoculated a laryngeal papilloma onto the human skin, obtaining papillomata on the arm and flat warts on the face and scalp.

It is therefore evident that, in considering the pathology and etiology of genital warts, all types of warts and even papillomata must be considered.

Histology.

Papillomata are epithelial tumours which grow from the skin, mucous membranes and the ducts of glands. They are also found in the interior of cystic tumours (Kettle 1925 (21)). They are usually simple but they may become locally malignant in

certain/

Fig.2. Genital Wart X 7. Mann's Stain.

Showing the branching processes with a connective tissue core covered by epithelium. (See colour photograph).

certain situations as in the bladder and larynx (Muir 1936)(31).

Although they are derived from epithelium, papillomata all contain connective tissue which grows along with the epithelium. A papillomata therefore consists of an outer covering of several layers of epithelial cells, which constitute the essential part of the tumour, and an inner core of vascular connective tissue which acts as a support and a path by which the blood supply can reach the cells. As the epithelium proliferates, it is necessarily thrown into folds in order to maintain its normal relationship to the underlying connective tissue. Thus many branching processes are formed.

All papillomata have these essential characteristics but they are modified by the original type of the epithelium. Probably other factors such as irritation, trauma and the blood supply to the affected part may cause slight variations. (Schofield (1937)(36). Thus in plantar warts pressure forces the interpapillary processes inwards so that the corium becomes invaginated and condensed.

The influences acting on genital warts are almost always the same. They are exposed to trauma but not to the same extent as a wart on the foot or hand. They are moist even if a discharge is not present. There is an abundant blood supply to the parts/

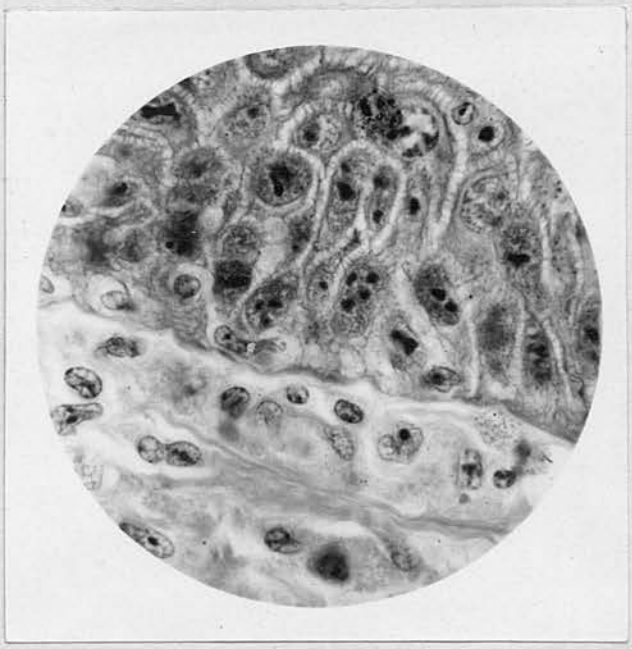

Fig.3. Genital Wart X 1000. Mann's Stain.
Malpighian layer of columnar cells.

parts affected and this is not likely to be diminished by cold as on the more exposed portions of the skin.

Although there may be marked variation in the size of genital warts, the histological picture remains very constant. The centre of the wart is composed of interpapillary projections of connective tissue which are prolongations of the normal sub-cutaneous tissue. They are similar to it in structure except that they contain numerous thin walled blood vessels and are much more cellular. The connective tissue is bounded by the cells of the Malpighian layer which retain their columnar shape but are larger than normal.

External to these lies the rete malpighii or prickle cell layer of polyhedral cells. This layer is several cells thick. As they get further from their blood supply, these cells begin to show various degenerative changes. They are seen in the nucleus, where the chromatin begins to collect into several small aggregations, and in the cytoplasm which becomes vacuolated. In the outer layers, where the changes are most marked, the cytoplasm may be entirely absent in paraffin sections and the degenerated nucleus appears to lie in a clear space surrounded by the cell wall.

These changes are not marked in the normal skin. (Sharpey-Schafer 1920)(37). They probably occur in warts/

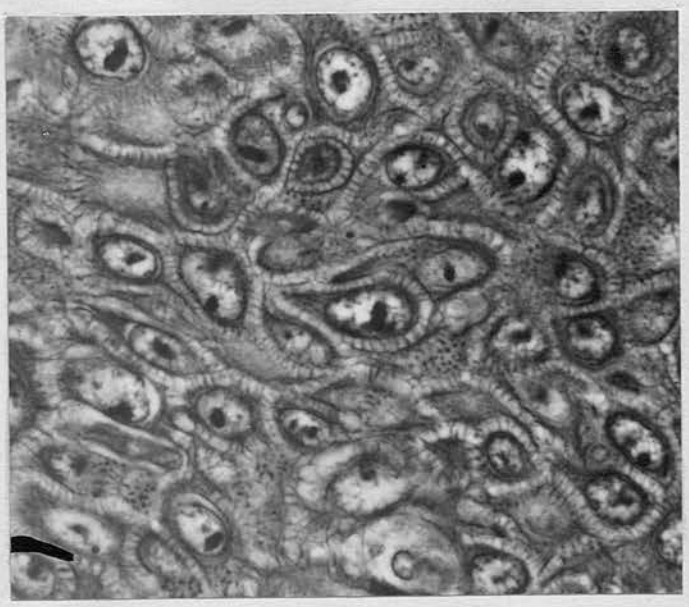

Fig.4. Genital Wart X 1200. Kull's Stain.

Prickle cell layer.

warts owing to the more rapid proliferation of the Malpighian layer. As a result, the consequent increased thickness of the prickle celled layer leads to a diminished blood supply to the outer cells.

The stratum granulosum forms the external layer. It is composed of cells which have undergone the final process of degeneration to form keratohyaline. These cells are full of granules and extruded nuclear material. (Ludford 1924-25)(27). This appearance is not to be confused with inclusion bodies due to a virus, which in warts occur in the deeper layers. The thickness of the stratum granulosum varied considerably in different cases but was not generally a marked feature of genital warts.

Melanin was present in the epithelial layer of the warts from Indian patients and also in a few of those from Europeans.

The histological appearance described may be considerably modified by a secondary pyogenic infection. This causes marked cellular infiltration of all the layers and oedema of the connective tissue. An increase in the cells of the connective tissue was also noticed in two warts of the previous series which had been treated by X-rays. In these there were many eosinophils and a few basophils.

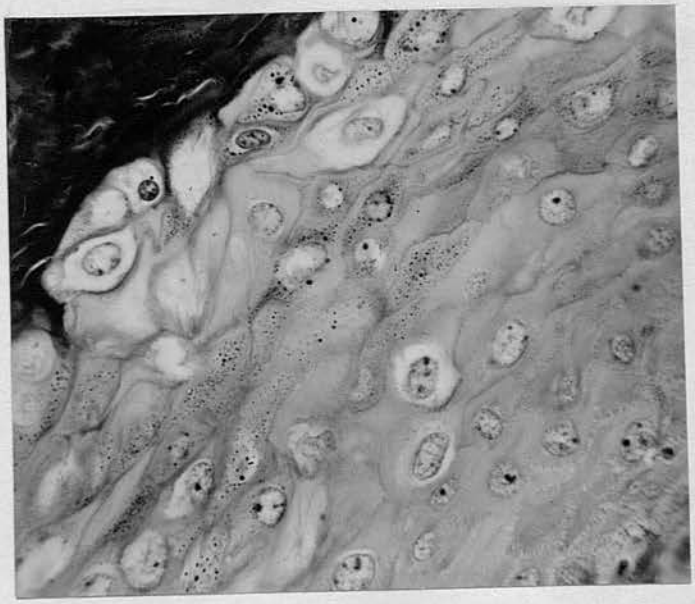

Fig.5. Genital Wart X 1000. Mann's Stain.

Outer layers showing the granules due to the formation of Keratohyaline.

Aetiology.

Previous theories:-

The histology of the condition is essentially the same as that of all other papillomata. There is therefore nothing in this to support the older aetiological theories which apparently were based mainly on clinical observations. These naturally laid most stress on the venereal infections as the cause of the condition.

Some confusion seems to have existed between genital warts or condylomata accuminata, and the condylomata lata of syphilis. However, other spirochaetes than the spirochaeta pallida were also described as the causal organism. Schaudin and Hoffmann (18) claimed to have found spirochaeta refringens in sections. Civatte and Favre (11) described intracellular and extra cellular "spirochaetal tufts" which were not caused by either spirochaeta pallida or spirochaeta refringens. These theories have never been confirmed. No spirochaetes were found in any sections from the present or previous series of cases.

It was most commonly considered that the warts had some connection with gonorrhoea as the name gonorrhoeal wart indicates. (Kettle 1935 (21), Muir 1936 (31), Kerr et al (1933) (20). In 1913 Armstrong/

Armstrong (6) claimed to have cured a case of gonorrheal warts of the face with a gonococcal vaccine, while Boyd (1938)(9) in his textbook states that they are venereal in origin and almost always due to gonorrhoea. Although such a direct connection with the gonococcus was not generally accepted, most of the text books stated that the warts were indirectly due to gonorrhoea and probably connected in some way with the irritation due to the discharge (Harrison (17).) As it has been noted already clinically, neither a discharge nor gonorrhoea is a constant factor in the production of the warts, nor do they exert a constant influence upon the course of the disease.

According to Frey about two thirds of his patients with genital warts were free from other disturbances of the genito-urinary tract. In the present series of nine cases only one had a concurrent gonococcal infection and three others a non specific balanitis.

Genital warts have therefore no connection with the gonococcus, spirochaeta pallida or similar organisms.

Present theory of the Etiology.

Warts in general, including those on the genitals,/

genitals, are now classed among the virus diseases.

There are at present two theories as to the nature of virus diseases. The cause may be minute living organisms capable of reproduction or auto-catalytic substances derived perhaps from the host's cells and capable of synthesising a similar substance (Findlay 1937 (14), Merlowes 1938 (30), Rivers 1938 (34).)

Heavy proteins have been obtained in crystalline form from various virus diseases of plants, and similar proteins have also been isolated from rabbit papillomata as well as equine encephalomyelitis and yellow fever. Although Jacobson and Jones (1938)(19) state that warts are probably due to a crystalline substance, there is some histological evidence that warts are caused by a minute organism rather than by some such protein enzyme.

Such organisms or Elementary Bodies have been described in many virus diseases including variola, vaccinia, sheep and fowl pox, psittacosis, ectromelia, lympho granuloma inguinale, Rous sarcoma, filterable fibromata of rabbits and others. These organisms do not appear to be able to lead a separate existence apart from the cells which they infect. Their presence in such an infected tissue is usually indicated by finding intracellular Inclusion Bodies.

These are present in many virus diseases and the Negri bodies of rabies, the Guarnieri bodies of small pox, the Bollinger bodies of fowl pox and those of molluscum contagiosum and psittacosis are among those generally accepted as morphologically specific for each disease. The true nature of these inclusion bodies and their relationship to the elementary bodies was demonstrated by Woodruff and Goodpasture (40). They digested the lesions of fowl pox with trypsin leaving the inclusion bodies intact due to their lipoid envelope. After separation and washing they were ruptured and were shown to contain the elementary bodies previously described by Borrel in 1904. The disease was also reproduced by these bodies alone. These inclusion bodies are therefore probably virus colonies enveloped and held together by gelatinous material produced either by the cell or by the virus. (Bedson)(8).)

There are other types of inclusion bodies, mainly the intranuclear ones, which are only virus specific and not type specific. The main example of this type is the acidophile nuclear body. (Gye and Ledingham)(15).) Other intranuclear inclusions described by Bedson (8) are of two types. The inclusion may occupy the whole nucleus while the nuclear chromatin is arranged in irregular clumps round the periphery of the nucleus which/

which is separated from the inclusion by a clear space. In the other type the nucleolus is hypertrophied and the nucleus contains small basophilic inclusions.

Inclusion and Elementary bodies of these types will be described from sections taken from genital warts. There is therefore no necessity to consider the demonstration of the ultramicroscopic type of virus in these warts.

The filter passing property of viruses depends entirely on the size of the filter. This was demonstrated by Elford (1931)(12) who used filtration through graded collodion membranes to measure the size of the various viruses. In studying the etiology of warts, filtration has been used mainly in the experimental transmission of the lesions to man. This ensured that any warts produced were not due to cell transplants. The filterability of the virus of warts was first proved by Ciuffo (1907) using a Berkfeld N candle and confirmed by Serra (1908) who used a Berkfeld W candle. Subsequent work by Wile and Kingery (1919)(39), Kingery (1921)(23) and Findlay (1930)(2) also showed that the causal agent could be filtered even through a Berkfeld V candle. It should now be more satisfactory to separate the virus by means of the high speed centrifuge.

Although two of the original conceptions of viruses/

viruses as filterable and ultramicroscopic may no longer be properly applied, there are several other properties which require to be considered.

As the viruses may not lead an existence separate from cells, tissue culture has been used to obtain cultures and to study their behaviour. Most of the common viruses have been propagated in this way and have been shown to have undergone active multiplication. (Parker (1938)(32).) The causal virus of warts has not been grown in this manner but attempts have been made to cultivate it on the chorio-allantoic membrane of the developing chick embryo.

The characteristic of species specificity possessed by many viruses has also been studied by means of tissue cultures. Thus fowl pox, which is innocuous for mice and rats, will not multiply in cultures of their tissues.(Findlay, 1928 (13).) A similar specificity is exhibited by fowl plague and the virus of foot and mouth disease. This may explain the difficulty found in transmitting warts to animals. Serra (1924) and Findlay (1930)(2) were both unsuccessful in this and the present series of experiments also gave no positive results. Ullman (1923) however, was successful in transmitting a laryngeal papilloma to the vagina of a bitch. Further evidence of this species specificity is given/

given by the warts which occur naturally on dogs and cattle. As in man filtrates of emulsions from these warts proved infective for other animals of the same species. But neither the canine nor the bovine warts could be transmitted to man, rabbits, mice nor guinea pigs. (Findlay (1930)(2)).

Although the transmission of warts to animals is difficult or impossible, it is relatively easy to infect man. For warts, like other virus diseases, are infectious as has already been discussed clinically. The experimental evidence of this has been summarised by numerous authors (Findlay,1930)(2), Goodman & Greenwood,(1934)(4), Brain(1933)(1) and Fine (3)). Following Variot, who in 1893 innoculated an adult from a child, Tadasshon (1896), Lanz (1899) and Tuliusberg (1903) all reported successful series of cases. This work was repeated and confirmed with cell free filtrates by a large number of workers including Cuiffo (1907), Serra (1908), Wile and Kingery (1919), Kingery (1921) and Findlay (1930)(2). In addition Findlay confirmed the earlier work of Waelsch and Serra already mentioned as well as that of Wile and Kingery (1919)(39).

There is thus little doubt that the various types of warts and probably also laryngeal papillomata are produced/

produced by the same agent which is filterable through a Berkfeld V candle.

The incubation periods observed in the experiments already mentioned varied considerably. Wile and Kingery (39) induced lesions in four weeks and Lanz and Tuliusberg recorded periods of six and seven weeks respectively. But Serra and Kingery found that the warts only appeared after about six months. The more recent experiments by Findlay (2) in 1930 produced periods varying from three to six months.

That there must be some type of immunity reaction to warts is shown by their well known property of disappearing spontaneously and by the fact that some people will not react when innoculated with wart extracts. Also Findlay (1930)(2) found that he became immune after being innoculated with three crops of warts. Whether this immunity is cellular or humoral is not known. At present little work has been done on this subject generally. In 1929 Andrews (5), using Virus III infection of rabbits, cultured in rabbit testicle in vitro, showed that there appeared to be a humoral immune factor. But Rivers, Haager and Mukenfuss (1929)(33), working with vaccinia also in tissue culture, obtained results which seemed to prove that the cellular factor was decisive. There is thus no definite experimental evidence/

evidence relating to the nature of the immunity reaction to viruses generally, and none with regard to warts.

Clinically, Brain (1937)(10) was unable to demonstrate the presence of antibodies in patients' serum and had no therapeutic success from injecting vaccines. He came to the conclusion that the spontaneous disappearance of warts was more likely to be the result of an abiotic change in the virus than the result of the action of specific antibodies.

However, a serum has been obtained which will neutralise the Shope papilloma. Also the antigen which binds complement when mixed with this serum is closely related to the virus itself and may be identical to it (Kidd (1938)(22)).

EXPERIMENTAL WORK.

The experimental work carried out on genital warts was commenced during the previous investigation and continued during this present series of experiments. The following experiments were carried out:-

1. Inclusion bodies were described in genital warts but could not be found in papillomata from the tongue, cheek, hard palate or a 'paraffin wart'.

2. Methods of demonstrating the presence of the virus by innoculating different laboratory animals on various sites were investigated.

    (a) Intracerebral innoculation of guinea-pigs having previously proved unsuitable, the intracerebral innoculation of mice and rabbits was attempted.

    (b) Intratesticular injection of rabbits was carried out.

    (c) Intradermal injection of rabbits was tried.

    (d) Intracorneal innoculation of guinea pigs was then investigated.

    (e) Finally a monkey was tested intra-cutaneously and subcutaneously in the anal and vaginal regions.

INCLUSION BODIES IN GENITAL WARTS.

In epidermal tissues the formation of Keratohyaline leads to degenerative changes and the appearance of homogenious granules of various sizes in the cells. (Ludford 1924-24 (27)). Unless care is taken, these may be confused easily with virus inclusion bodies.

Thus Findlay (1930)(2) summarised the previous work on these inclusions. He considered that, of the acidophilic cytoplasmic inclusions described by Sangiorgi (1915) and by Lipschütz (1924), those described by Sangiorgi are almost certainly nucleolar extrusions. Also Lee (1933)(25) produced by chemical means various intra-nuclear inclusions. This may be the explanation of the intra-nuclear inclusions described in warts by Sanpelice (1913), Lipschütz (1924) and Ullmann (1923) which Kyrle (1925) regarded as oxychromic changes of the nucleoli.

The inclusion bodies described here do not occur in the outer layer of the epidermis, where keratohyaline is being fofmed, but in the deeper layers, and in the Malpighian layer itself. None were found in the connective tissue. They are never very numerous although several often occur together in a small area./

Fig.6. Genital Wart X 1000. Mann's Stain.

Outer layers with a large "inclusion" probably due to the formation of keratohyaline. This is for comparison with figures 7 and 8. (See colour photograph).

area. These inclusions are of two types - cytoplasmic and intranuclear.

The cytoplasmic inclusions are spherical in outline. In sections stained by Mann's method they are usually eosinophilic although their staining reaction is sometimes irregular. This has been noted in the inclusion bodies of other diseases. The inclusions are packed with small refractile granules. These may be the elementary bodies as they closely resembled those of fowl pox.

This type of inclusion body is found in the cytoplasm of the cell sometimes causing the nucleus to become indented. More often the inclusion is lying free surrounded by a clear zone. It appears that the cytoplasm of an infected cell undergoes degeneration. This, in a paraffin section, leaves the inclusion and the cell nucleus lying free surrounded by the cell wall. Ludford and Findlay (1926)(28) consider that the formation of a small vacuole is the earliest indication of the infection of an epidermal cell with fowl pox. It is possible that the cell changes in warts are the end result of a similar process.

The intranuclear type of inclusion is found either in association with a cytoplasmic inclusion or in a cell whose cytoplasm is apparently normal. Some nuclei may be found with a swollen nucleolus and the/

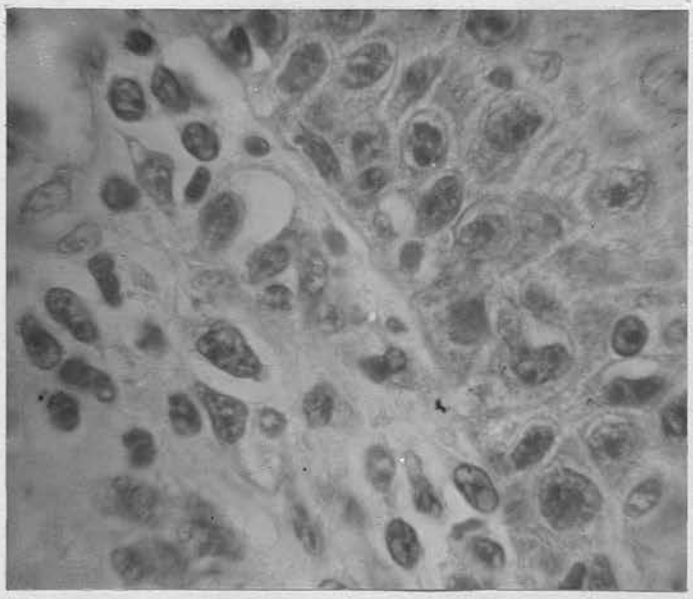

Fig.7. Genital Wart X 750. Mann's Stain.

Columnar cell layer with a cytoplasmic inclusion body. (see colour photograph.)

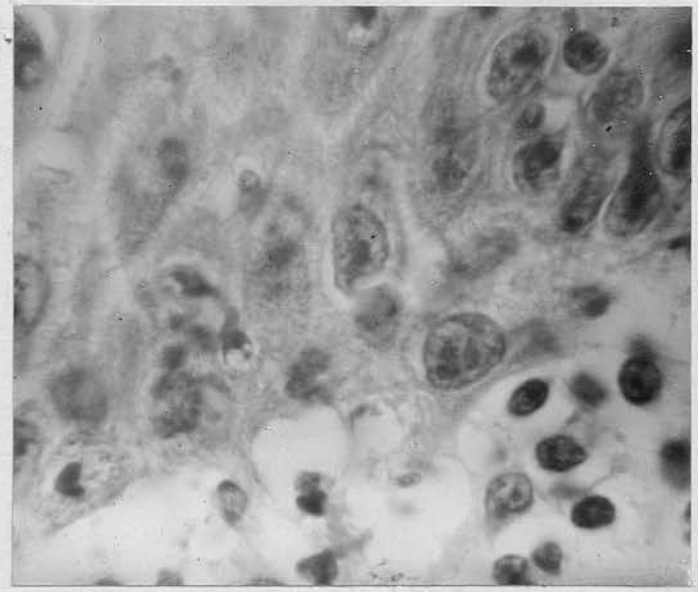

Fig.8. Genital Wart X 1200. Mann's stain.

Columnar cell layer with a cytoplasmic inclusion body. (see colour photograph).

the chromatin grouped round the nuclear membrane. These are probably degenerative changes as they are often seen in cells containing a cytoplasmic inclusion. The more typical intranuclear inclusion is only present in a few cells. In these almost the whole nucleus is occupied by a spherical acidophilic body. This is separated by a clear space from the chromatin which is found in irregular clumps round the nuclear margin.

Other papillomata were also examined for the presence of inclusion bodies. None could be demonstrated in sections of papillomata from the tongue, hard palate and cheek. Nor could any be found in sections from a "paraffin wart".

INTRACEREBRAL INNOCULATION.

In the present series of experiments it was decided not to use the intracerebral method of innoculation. Goodman and Greenwood (1934)(4) describe a series of experiments by Dienes and Kubik. They obtained negative results on injecting ground-up wart material into the brains of eight guinea-pigs. In the previous investigation, two similar series of experiments, one on mice and one on rabbits, were carried out also with negative results.

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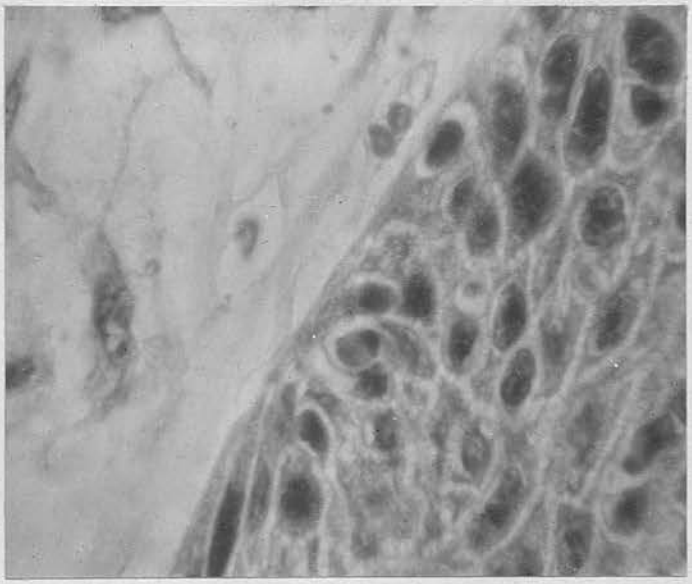

Fig.9. Genital Wart X1000. Mann's stain.

Columnar cell layer with an intra-nuclear inclusion body and irregular clumping of the nuclear chromatin. (see colour photograph).

In the first series six mice were injected intracerebrally with 0.2 cc. of wart extract. Three mice received similar injections of autoclaved extract as controls. Part of the brain from one of the six test mice was removed, extracted and injected intracerebrally into three more mice. On histological examination of the brains nothing was found which was in any way suggestive of a virus infection.

In the second series two young rabbits each received 2 cc. of the wart extract intracerebrally. No effects were seen in either.

From these facts it was concluded that the intracerebral injection of guinea-pigs, mice and rabbits could not be used as a method of demonstrating the virus.

INTRATESTICULAR INJECTION.

In the previous investigation 0.5 cc. of an extract of a wart, which had been stored in 50% glycerine for 10 days, was injected into the body of the testis of a young rabbit. An intense reaction took place within two days. The testis became enlarged to about three times its normal size then commenced to subside slowly. Half of this testis

was/

was extracted as before. The extract was injected into the testis of another young rabbit and the brains of three mice. This time the results were negative.

Sections from the other half of the first testis showed increase of the interstitial tissue and degeneration of the tubules. Many of the cells contained eosinophil granules but these were probably degeneration products.

As it seemed that this might be a convenient method of demonstrating the virus, the following series of experiments were carried out in the present investigation.

A wart was removed under local anaesthetic from the coronal sulcus of a case of genital warts. It was placed in 50% glycerine saline for thirty days. A small piece of normal skin removed at operation on another patient was treated in exactly the same way for use as a control.

At the end of the month the two pieces of tissue were washed in saline then ground in a sterile mortar and extracted with normal saline. After being centrifuged at a slow speed to throw down any small pieces of tissue, the clear extracts from the wart and skin were each divided into two portions. One portion of each was autoclaved.

Four/

Four separate extracts were thus obtained; wart extract, autoclaved wart extract, normal skin extract, autoclaved skin extract. They were tested for sterility aerobically and anaerobically. The bodies of the left testes of eight rabbits were injected with 0.5 cc. of the extracts as noted below.

The testes were observed daily for fourteen days. No reactions were seen. Sections taken from testes injected with wart extract were all normal.

Rabbit No.	Material injected.	Result.
1	Wart extract	These were observed for fourteen days after injection. All were negative.
2		
3	Autoclaved wart extract.	
4		
5	Skin extract.	
6		
7	Autoclaved skin extract.	
8		

In view of the result obtained previously, this investigation was repeated using different rabbits and material from a common wart on the hand. Exactly the same conditions were observed except that/

that the wart was kept in 50% glycerine saline for only fourteen days.

Eight injections were carried out and the rabbits were observed daily for fourteen days. The results obtained were again all negative.

Rabbit No.	Material injected.	Result.
21	Extract from a common wart.	After fourteen days observation all were negative.
22		
23	Autoclaved Wart Extract.	
24		
25	Normal skin extract.	
26		
27	Autoclaved Skin extract.	
28		

A final experiment was carried out using a small genital wart removed from the coronal sulcus. It was stored in 50% glycerine saline for sixteen days. It was then ground as before but not centrifuged. This time the whole of the material was taken and it was well mixed before injection. 0.5 cc. was injected into the body of the left testicle of full grown rabbits.

In no case was any reaction noted.

Rabbit No.	Material injected.	Result.
29	Whole wart extract	After fourteen days there was no reaction.
30		
31	Autoclaved wart extract	
32		

INTRADERMAL INJECTION.

Previously varying amounts of sterile wart extract were injected intracutaneously and subcutaneously into the back of a rabbit. Nothing abnormal was noted. In spite of the negative result obtained this was repeated in the present series.

The material used was that prepared for the first series of testicular injections. Four rabbits were injected intracutaneously with 0.2 cc. of wart extract as follows. The results were again all negative after five months.

Rabbit No.	Material injected.	Result.
9	Wart extract.	Nothing noted after daily examination for five months.
10		
11	Autoclaved wart extract.	
12		

P. conjunctivae

INTRACORNEAL INNOCULATION.

A wart was removed under local anaesthesia from the anal region of a case of genital warts. It was divided into two parts and used for two series of experiments.

Series I.

Half of the wart was immediately ground in a sterile mortar and extracted with normal saline. This extract was divided into two parts one of which was autoclaved. These two extracts were then injected into the conjunctiva of the lower lids of three guinea pigs. Injections of 0.05 cc. were used in each case.

The animals were observed for six months. Nothing abnormal was seen.

Guinea pig No.	Left eye.	Right eye.	Results.
1 2 3	0.05 of the wart extract.	0.05 cc. of the auto-claved wart extract.	Nothing noted after six months.

Series II.

The other half of the original wart was stored in 50% glycerine saline for fourteen days. It was washed, ground and extracted with normal saline. The extract was divided into two portions, one being autoclaved as before. A similar series of injections were carried out as in Series I. No change was observed within six months of injection.

Guinea pig No.	Left eye.	Right eye.	Result.
4) 5) 6)	0.05 cc. of the wart extract.	0.05 cc. of the autoclaved wart extract.	Nothing seen six months after injection.

MONKEY INNOCULATION.

Part of the wart extract which had been stored in 50% glycerine saline for fourteen days and which was used in the guinea pig innoculation was taken for injection into a young female monkey. 0.3 cc. were injected intracutaneously and subcutaneously into the skin around the anus. A similar injection was made into the skin at the entrance to the vagina. The monkey was observed for four months but nothing abnormal was seen.

DISCUSSION.

In the past there has been much confusion about Genital Warts. They appear to have been named, described and treated according to individual opinions unsupported by any definite facts. No doubt this is because they are usually a complication of some more important disease.

From clinical observation very few facts emerge. It is clear that they are warts modified and distinguished from common warts only by their anatomical position. This makes 'genital wart' the most appropriate name for the condition. They are infectious and due to their position this infection is often venereal, but the infection may just as easily be from another source such as an autoinfection from a wart on the hand.

The statement found in many books that they are a result of a discharge is incorrect, although there seems to be no doubt that a discharge may modify the warts. Another fact of importance is that, when they are present on pregnant females, they increase enormously even during the first months and regress rapidly after parturition. Beyond this, very little is known about the clinical condition and the factors which influence it.

However/

However, the main interest in genital warts lies in their etiology. In structure they are papillomata. And there is no essential difference between them and common warts (Beattie and Dickson 1925(7)). This has not always been realised in the past and their histology has been described separately from other papillomata. Not only is their histology similar but genital warts, common warts and laryngeal papillomata may be produced by innoculating the same material into different sites. Thus common warts have been obtained by injecting into the skin material from genital warts and laryngeal papillomata (2,3,4.) These conditions can not be separated when their etiology is being considered. The problem therefore concerns not simply a relatively unimportant group of benign papillomata, but also other papillomata which may become locally malignant and perhaps all papillomata.

Though genital warts, common warts, and laryngeal papillomata all appear to be caused by the same agent, only the laryngeal papillomata may become locally malignant. There is nothing to indicate a reason for this except the difference in the original character of the epithelium affected. It would seem that mucous membranes are more liable to undergo malignant changes than is the skin, for the causal agent/

agent appears to be the same for both the benign and malignant tumours. It is impossible, however, to draw any definite conclusions from this at present.

The older theories of the venereal, gonorrhoeal or syphilitic origin of genital warts did not take into account this relationship between the various types of papillomata, although this had been indicated as early as 1900 by the clinical observations of Rasch (2,3,4.) Apart from omitting to explain this essential fact there has never been any evidence of a direct connection between genital warts and any venereal disease, though frequently they may be found in association with a concurrent disease of venereal origin. Therefore these diseases need no longer be considered as etiological factors in this condition.

Many chemical agents lead to the production of cancer. The oestrogenic hormones belong to this group. It is necessary that they should be considered with regard to genital warts owing to the changes observed in the size of the warts during pregnancy. As moisture has no such effect on warts in males and as the blood supply to the part could not be greatly affected as early as the second month, it appears that the cause of this variation in size must be some factor directly connected with the pregnancy. Of these the most likely seems to be a hormone.

Lacassagne/

Lacassagne (1936)(24) has shown that an increase of ovarian secretion in mice leads to the occurrence of mammary carcinoma. Here, however, the hereditary factor and large doses given over long periods were necessary before any results could be obtained. The changes in genital warts correspond almost directly to the changes in the ovarian secretion. But the warts do not become malignant nor is there any evidence that pregnancy predisposes to their occurrence. Also, unlike the carcinomas in mice, the process is reversible for the warts decrease rapidly in size after parturition. Therefore it would appear that these changes in size are not a result of the cancer producing effect of the oestrogenic hormones but rather a result of the general hyperplasia which they cause in many organs including all cutaneous tissues. It is thus unlikely that the oestrogenic hormones play an important part in the etiology of these warts.

The large number of successful experiments quoted prove that these tumours may be transmitted from case to case by means of a cell free filtrate. Cell transplants are therefore not the responsible agent in the spread of the disease. These experiments also exclude the factor of mechanical irritation. The irritation of discharges has often been quoted Harrison (17) in the past as the cause of genital warts/

warts.

It has been shown by clinical evidence alone that this cause may be excluded. The experiments in transmission prove this beyond doubt, for the warts have been reproduced on other parts of the body where these discharges cannot exist.

Warts are at present grouped among the virus diseases. This was done on the assumption that the causal agent was a living organism of the type found in molluscum contagiosum or fowl pox (1,2,3,4). There is some evidence to support this theory. But, when the position is considered in the light of research into the nature of tumours generally, it will be seen that this has not been conclusively proved. It appears that there is not enough known about the viruses responsible for growths to be certain about their nature. (Hadfield & Garrod, 1938 (16)). Heavy proteins have been isolated from rabbit papillomata and Jacobson and Jones, 1938 (19) consider that warts are probably due to a similar crystaline substance. There is also some indication that a living organism is the cause. At present, therefore, the exact nature of the causal factor must remain in doubt, so that the evidence will be considered without drawing any conclusions as to the type of "virus" agent present.

The facts in support of the theory that such a virus/

virus agent is the cause appear to be:-

(1) That these tumours are transmissible by means of cell free filtrates.

(2) That the infective agent will withstand fifty per cent glycerine.

(3) That these tumours seem to show marked species specificity.

(4) That inclusion bodies have been described in sections.

Of these, the first two, their transmissibility and their resistance to fifty per cent glycerine, may reasonably be accepted as proved. The question of species specificity and the presence of inclusion bodies require further consideration.

Warts have been reproduced so often using ground up material from lesions on the skin, genitals, and larynx and also later by means of filtrates of this material, that there can be no doubt as to their being transmissible. Similar experiments have been successful after the warts used have been stored in fifty per cent glycerine for varying periods up to two months. While these correspond to similar properties held by other virus diseases caused by living organisms, they do not exclude an inanimate substance.

As/

As has already been stated, some viruses have a property of being specific for one species. The negative results obtained in the experiments carried out in this series of investigations on guinea pigs, rabbits and monkeys and the previous unsuccessful attempts to reproduce the lesion in mice would appear to show that this virus, if present, is also markedly specific for one species only. This is in agreement with previous work. Because, with the exception of the successful innoculation of material from a human wart into the vagina of a bitch, all attempts to transmit the lesions to laboratory animals have failed. Also just as warts present on humans may be transmitted to animals of the same species, and just as human warts are specific for man only, so bovine and canine warts are specific for cattle and dogs only (2).

It would seem that this corresponds to the species specificity of the viruses of other diseases. But it would mean that there must be either a separate virus or a separate strain of the same virus for each species. Unfortunately antibodies to the virus have not been demonstrated. (2,10). While this is in itself an argument against a virus as the causal agent of warts, it also prevents any tests of the relationship between the tumours present on the different/

different species being carried out by means of the neutralising effects of the various sera.

The Shope papilloma occurring in rabbits must now be considered, for this is also a papilloma which has the same characteristics as those in man and other animals. It may be transmitted by means of a cell free filtrate and under certain conditions may become malignant. In addition it shows a marked species specificity for the rabbit and even for one type of rabbit, the cotton tail. Antibodies to this tumour were formed in the rabbit. The rabbits became immune to reinfection, as Findlay (2) did after innoculation with three crops of warts, and the serum from these rabbits inactivated the virus (22,35). If it could be shown that this serum would inactivate the filtrate from papillomata from man and other animals, it would prove a definite relationship between these tumours.

The presence of inclusion bodies in other virus diseases has already been discussed. The inclusion bodies which have been described here closely resemble these. It is clear that they are quite different from the acidophil bodies due to the formation of keratin which are seen in the cells of the stratum granulosum. It only remains to consider the significance of this. If these bodies are constantly found/

found in all warts, they constitute a very strong support for the theory of a living virus as the cause of the condition, for these inclusions are packed with spherical bodies which appear to be the virus particles themselves. That these inclusions are not numerous and are not found in every section is similar to what may be found in typical virus diseases such as rabies. The Negri bodies are specific for this condition. But only a few were found after many sections had been examined from the hippocampal region of a case, although the man, a soldier, died after a typical attack of rabies with all the neurological symptoms. It is therefore not necessary for the inclusion bodies to be present in any great numbers as may be found in some other virus diseases.

Though these typical inclusions are present, it does not necessarily follow that the virus which causes them also causes the warts. Rivers found that rabbit tumours could contain virus III and vaccinia virus and that these viruses could be carried in the tumour throughout a series of transplants (16). So that although these inclusion bodies are strong presumptive evidence that a living virus is the causal organism, they are not conclusive proof of this.

When all the facts are taken together, it seems that some type of virus is present. But a final

decision about this can only be obtained when research into tumours in general has progressed further. In this progress the study of genital warts may prove to play an important part.

SUMMARY AND CONCLUSIONS.

1. The clinical condition is described with special reference to etiology.

2. The similarity in the histology of all papillomata is noted. The connection between genital warts, common warts and laryngeal papillomata is considered.

3. These papillomata are examples of a transmissible tumour in man.

4. These tumours are similar to the Shope papilloma of rabbits.

5. The older and more recent theories of the etiology are discussed. It is concluded that these warts are probably caused by a virus the nature of which must remain in doubt at present.

6. Attempts were made to transmit the lesion to animals. It was found that mice, guinea pigs, rabbits and a monkey were all unsuitable, probably owing to the species specificity of the virus.

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